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SECOND ANNUAL REPORT

February 1, 1993 - January 31, 1994

"Evaluation of Dried Storage of Platelets for Transfusion:
Physiologic Integrity and Hemostatic Functionality"

Grant No. N00014-92-J-1244
ECU Grant #5-00752
From the Office of Naval Research:
Navy Medical Research and Development Command
Department of the Navy

Performance Sites and Investigators:

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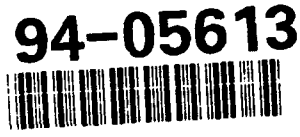

Worksite Activity Review:

The work has been proceeding as outlined in the proposal. At UNC-Chapel Hill, the main focus has been on studies in animals, including whole body scans in dogs after infusion of radiolabelled rehydrated canine platelets and assessment of multiple infusions of non-labelled rehydrated platelets. The attached subcontract report from Dr. Read and colleagues shows that the radiolabelled platelets were distributed in the dog(s) in a diffuse pattern when examined 4 hours after infusion. This is a good result, considering that a finding of localized accumulations of infused platelets might have indicated a tendency for spontaneous thrombus formation. The multiple infusion studies have not produced any evidence as yet of an immune reaction to rehydrated homologous platelets (up to 7 infusions in same animal); however, earlier experiments showed that is was necessary to change from bovine serum albumin in the preparation to canine albumin to avoid an immediate allergic reaction.

At the ECU worksite, we greatly intensified efforts to test the in vitro responsiveness of human rehydrated platelet preparations for activation and adherence in a Baumgartner-type perfusion chamber. Since installation of the Virtis 600 freeze-drier at ECU on 9/22/92, we have made forty human platelet preparations (and also thirteen canine preparations for a related ONR project). Of the 32 preparations produced in this annual reporting period (up to 12/16/93), 13 were to explore alternative stabilization strategies (permanganate at 0.002 - 0.02%) while the others were done with paraformaldehyde at 45, 60, or 120 minutes. The activation and adherence data favor continued development of the use of paraformaldehyde for 45-60 minutes as the primary stabilizer instead of permanganate. Two permanganate platelet preparations were entered into a long-term storage study (aliquots placed in dessicators at room temperature, 4°C, and -70°C) on 8/31/93 to see if "perm-platelets" have an advantage over the stored "para-platelets" reported on below.

Six para-platelet preparations were processed entirely in the blood bag collection pack to simulate the closed system and sterile processing that will be necessary for pharmaceutical production. In two of these preparations, the native plasma was used for washing between processing steps to make the system even more "closed". Other such practical developments will be pursued by collaborators Armour Pharmaceutical Corp. The data presented in this report from the ECU worksite pertain to the testing of all these human platelet preparations in the Baumgartner perfusion chamber.

Patents and Extramural Collaboration:

A continuation-in-part of the original patent application was filed on 4/28/93 in the U.S. Patent Office. Filing has also been made under the Patent Cooperation Treaty (# W093/23997, publication date 12/9/93). There are still some unresolved questions impeding final award: are prior project reports to the ONR considered open and enabling publications, and how do the current preparations differ from the prior, patented preparations by Brinkhous and Read. "Brinkhous preparations" were included in the Baumgartner perfusion experiments detailed below for comparison to present preparations for this reason.

The original workplan included a specific aim (#4) to measure the metabolism of rehydrated platelets in a collaborating laboratory at ECU or elsewhere. This effort

has not been carried out to the satisfaction of the principal investigator. Therefore, the remaining resources in this part of the budget will be utilized in a new direction; to study the effectiveness of rehydrated human platelets in correcting the bleeding time in thrombocytopenic rabbits according to the model of Morris Blajchman (see attached letter). This change in direction should yield valuable information on the hemostatic potential of rehydrated platelets to corroborate our other experiments. The metabolic function of these platelets will be at least partially explored by analysis of thromboxane production, as reported in the Baumgartner perfusion experiments.

On 8/20/93, a site visit and collaborative review session was conducted by Armour Pharmaceutical Co. to initiate a cooperative effort for large scale production of sterile lyophilized platelet preparations. Scott Murphy, M.D., of the Cardeza Foundation was brought in as a technical consultant. All relevant data was presented by the project PI and Co-PI and plans were evolved to set-up plateletpheresis processing centers with Armour's subsidiary Plasma Alliance Corp. for some initial consideration of logistics. These plans are now going forward with the additional support of ONR funds to Armour. The PI and Co-PI will act as consultants.

Scientific Progress:

We have found that perfusion of denuded, everted canine arterial vessel strips with rehydrated platelets in reconstituted whole blood provides an excellent model system for testing two important aspects of platelet physiologic function: adhesion to a thrombogenic surface, and recruitment of circulating platelets to activate. In this system, either fresh, unfractionated whole blood in citrate is recirculated over the vessel strip in a Baumgartner chamber, or rehydrated platelets are combined with washed RBC and platelet-free plasma to reconstitute whole blood for recirculation. In all of our Baumgartner-type perfusion experiments, we have attempted to quantify the adhesion capability of rehydrated or fresh platelets by morphometric examination of the vessel surface after incubation with a fluorescent antibody to the platelet marker GPIIbIIIa (P2 MoAb). The result was computed as a percent of the vessel surface covered by fluorescent cells (platelets), as given in Table 1. In addition, assays were performed on samples of the reconstituted blood immediately before or after the perfusion to look for evidence of activation in the nonadherent platelets: increase in CD62 or GP53 neoantigen expression on the platelet surface (by flow cytometry), or increase in levels of released Thromboxane B_2 (by radioimmunoassay). Cumulative results presented in Tables 2 and 3 compare the results of standard para-platelets to those of "Brinkhous platelets" (stabilized with 1.8% paraformaldehyde for 2 hours instead of 45-60 minutes in the para-platelet preparations), platelets stabilized with permanganate (0.002-0.02%), and platelets from liquid concentrates after expiration from the blood bank inventory. The expired PC are know to have defects in adhesion and activation [see reviews in Vox Sanguinis Vol. 40, Supplement 1, 1981] and thus provide a good negative control. A more pertinent comparison was that of para-platelets versus Brinkhous preparations or perm-platelets, which show that the newly developed para-platelets have both adhesion capabilities and an activation response in this system, as opposed to other preparations.

Table 1. Adhesion of platelet preparations to everted vessel strips in the Baumgartner perfusion chamber.

Preparation	Percent Coverage of Vessel
Rehydrated para-plts (n=6)	59% ± 16
Rehydrated Brinkhous-plts (n=4)	58% ± 19
Rehydrated perm-plts (n=9)	43% ± 19
Expired liquid PC (n=3)	37% ± 27
Fresh platelets (n=32)	73% ± 14

In each test of adhesion of rehydrated platelet preparations, a fresh platelet control was also run. By paired t-test the para-plts and Brinkhous-plts were not different ($p \geq 0.1$) from the fresh platelets in percent coverage of the vessel surface. The expired PC platelets and permanganate-stabilized platelets were less adhesive than the fresh platelets ($p \leq 0.05$) under these conditions, and somewhat less adhesive that the para-plts. We conclude that the para-platelets performed adequately in this test of platelet adhesion.

The nonadherent recirculating platelts were assayed for evidence of activation after exposure to the denuded vessel under the assumption that this system provided a controlled test of responsiveness of rehydrated platelets to physiologic stimulation. Presented in Table 2 are the data from flow cytometry immunophenotype analysis for neoantigen markers of activation. Comparison of pre and post perfusion samples was made by paired t-test to detect significant increases in activation markers during the run in each group.

Table 2. Detection of activation neoantigens CD62 and GP53 on the surface of nonadherent platelets in the Baumgartner perfusion experiments:mean percent positive cells by flow cytometry.

	CD62		GP53	
Preparation	Pre	Post	Pre	Post
Rehydrated para-plts (n=6)	10%	17%*	10%	19%**
Rehydrated Brinkhous-plts (n=5)	27%	20%	13%	12%
Rehydrated perm-plts (n=4)	37%	54%	18%	12%
Expired liquid PC (n=3)	38%	42%	17%	14%
Fresh platelets (n=21)	12%	14%	8%	7%

paired t-test * $p = 0.03$, ** $p = 0.006$

The main finding was that the rehydrated para-platelets showed evidence of activation after perfusion even though they were stabilized with 1.8% paraformaldehyde for 1 hour in process. The other preparations did not show this level of active response or activatability; the numerical increase in CD62 for perm-platelets was not statistically significant ($p = 0.2$). The fresh platelets were inconsistent in giving evidence of activation, which we are further investigating with varied controls.

The increase in percent positives for CD62 or GP53 was corroborated by a perceptible change in the mean peak channel of fluorescence (Mpk) of the whole distribution for the rehydrated para-platelets: for CD62, the mean Mpk increased from 176 to 228, for GP53 the mean Mpk increased from 197 to 228. These increments did not achieve statistical significance by paired t-test ($p = 0.08$-0.12) because of large standard deviations in the group means (data not shown). The decrease in % positivity for CD62 in the Brinkhous platelets from 27% to 20% was actually significant ($p = 0.001$) and confirmed by a significant decrease in Mpk from 266 to 240 ($p = 0.003$).

Comparison across the groups of preparations showed other significant findings in independent t-tests. The Brinkhous platelets, perm-platelets, and the expired PC had higher % positives for CD62 than the fresh platelets before perfusion ($p < 0.01$), which was corroborated by higher Mpk (266 and 392 vs 225). The platelets had a greater % positivity for GP53 than fresh platelets only after perfusion ($p < 0.01$). None of the groups showed a significant change in % positivity of markers for GPIb or GPIIbIIIa pre/post perfusion ($p > 0.1$). Across the groups, the expired PC had a lower % positivity for GPIb (AN51 MoAb) than the fresh platelets pre-perfusion: 61% vs 83% ($p = 0.05$), but no other markers showed significant differences.

Assays for Thromboxane B_2 were also performed on the supernatant plasma taken from samples pre and post perfusion as further evidence of platelet activation. The generation of TxB_2 indicates activation of the eicosanoid metabolic pathway, producing strong recruitment messengers capable of activating nearby resting platelets.

Table 3. Thromboxane B_2 levels (pg/0.1 mL) in the supernatant plasma before and after perfusion of reconstituted whole blood with the listed constituent platelets in the Baumgartner perfusion chamber.

	Thromboxane B_2	
Preparation	Pre	Post
Rehydrated para-plts (n=7)	300 ± 75	1590 ± 1160**
Rehydrated Brinkhous-plts (n=2)	123 ± 79	154 ± 48
Rehydrated perm-plts (n=3)	490 ± 120	300 ± 66
Expired liquid PC (n=3)	250 ± 70	493 ± 183*
Fresh platelets (n=15)	68 ± 38	333 ± 236**

Paired t-test = * $p = 0.05$, ** $p < 0.001$

Here again our data show that the para-platelets seem capable of a significant activation response in producing thromboxanes after exposure to a denuded vessel strip. The expired PC and fresh platelets also showed a significant generation of TxB_2 in this system; however, the expired PC gave only a doubling in TxB_2 levels over the "pre" sample, while the para-platelets and fresh platelets had a 40-50 fold increase. Comparisons across the groups showed that the para-platelets produced a significantly higher TxB_2 level than the fresh platelets or expired PC ($p < 0.001$). There was considerable variation in TxB_2 levels of rehydrated platelet preparations before perfusion, but it appeared that the act of blood component separation and reconstitution with substitute platelets generated 100-200 units of TxB_2 just by activation in handling.

In summary, we have used the Baumgartner perfusion chamber to demonstrate that many of the rehydrated platelet preparations will adhere to denuded vessel strips nearly as well as fresh platelets. However, only the platelets from our recently developed paraformaldehyde stabilization process (1.8%, 1 hour, in 5% BSA) showed evidence of a metabolical activation response in expressing CD62 or GP53 on the surface and generating TxB_2. The platelets treated by previous paraformaldehyde protocol or our alternative permanganate process were metabolically inert and not responsive in the activation assays. The para-platelets also appeared to outperform platelets from expired blood bank concentrates. We conclude that the para-platelets possess the two hemostatic features evaluated in vitro in this phase of the project: adherence to subendothelium and physiologic activation response. We will continue to confirm this finding with investigation of appropriate controls, such as seeing if para-platelets adhere to non-thrombogenic surfaces (non-denuded vessel strips), and seeing what effect inhibitors of platelet activation have on the adherence or activation response of para-platelets. At this point, we are greatly heartened by the demonstrated properties and responsiveness of the para-platelet product of this research. Manuscript(s) are in preparation to publish these findings.

Long-Term Storage Experiments:

The results from 12 months of storage of para-platelets at room temperature vs 4°C vs -70°C were presented in detail in the second triannual report of this project year. In brief, we found that storage of lyophilized platelets at room temperature in a dessicator did not preserve GPIb or GPIV adequately (staining with MoAbs SZ1 and CD36 fell very significantly); also these platelets lost response to hypotonic shock and ristocetin-induced aggregation. Storage at 4°C or -70°C showed no such clumps over 12 months. Another long-term storage study has now been initiated with platelets stabilized in 0.02% $KMnO_4$. Similar storage studies are planned with the platelet products to be prepared by Armour Pharmaceutical. We hope to demonstrate a shelf-life of 2 years or more for pharmaceutical preparations of freeze-dried human platelets.

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- *SERVICE*
- *RESEARCH*
- *EDUCATION*

Dr. Arthur P. Bode
Department of Pathology and Laboratory Medicine
1S-08 Brody Medical Sciences Building
East Carolina University
Greenville, North Carolina 27858-4354

July 13, 1993

Dear Arthur:

I enjoyed the opportunity to talk to you at the recent ISTH meeting in New York City about the possibility of collaborating on evaluating the hemostatic efficacy of freeze-dried platelets and platelets that have been stored for several weeks. As promised, enclosed please find a recent manuscript which is presently in the final stages of the review process by **Blood**. I believe that it will be accepted for publication.

I look forward to hearing from you soon about this matter.

With warmest personal greetings,

Yours sincerely,

M.A. Blajchman, MD, FRCP(C)
Professor, Pathology and Medicine
Fax (416) 527-4866

Enc.

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Dr. Morris A. Blajchman
McMaster University
Department of Pathology
1200 Main Street West
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Canada L8N 3Z5

Subject: Subcontract Agreement

Dear Dr. Blajchman:

Dr. Arthur Bode of ECU's Department of Pathology and Laboratory Medicine has indicated an interest in establishing a subcontract agreement with you and McMaster University to test lyophilized platelets in an in vivo model. Accordingly, enclosed you will find two original copies of the required subcontract agreement.

If, upon review, the terms and conditions of the agreement are determined to be acceptable, then please have the appropriate individuals sign the agreements and return one fully executed copy of the agreement to my attention. Please feel free to call me if any discussions are required regarding the agreement terms.

Sincerely,

Martha J. S. Van Scott
Assistant Director

Enclosures

cc: Arthur P. Bode, Ph.D.

Greenville,
North Carolina
27858-4353

SUBCONTRACT REPORT FROM UNC-CHAPEL HILL

SECOND ANNUAL REPORT

February 1, 1993 - January 31, 1994

"Evaluation of Dried Storage of Platelets for Transfusion:
Physiologic Integrity and Hemostatic Functionality"

Grant No. N00014-92-J-1244
From the Office of Naval Research:
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Performance Sites and Investigators:

University of North Carolina at Chapel Hill
Marjorie S. Read, Ph.D., Robert L. Reddick, M.D., Co-Investigators

1993 Annual Report for Grant No. N0014-92-J-1244

1. The hemostatic and thrombogenic effects of the rehydrated platelets and the characterization of the surface antigens of rehydrated platelets (Specific aim #1)

Our studies on the hemostatic and thrombogenic effects of rehydrated platelets involve the use of thrombocytopenic animals. We plan to use a COBE Spectra Blood Cell Separator apheresis machine to generate thrombocytopenic animals, obviating the need for chemotherapy or irradiation of experimental animals. The use of these thrombocytopenic animals will eliminate variability in results due to chemical interactions and the poor health of the animals. Results from an initial attempt at the generation of a thrombocytopenic animal in this manner suggest that this method will be feasible for our purposes. During this experiment, the platelet count for the experimental canine was reduced from 300,000/mm^3 to approximately 50,000/mm^3 over a 2 hour period. Bleeding times for the canine increased from 2 minutes at the beginning of the experiment to 4 minutes at the end of the experiment. The canine was kept under general anesthesia during the experiment. We anticipate using this procedure in the future for successfully generating thrombocytopenic animals without using anesthesia.

We have also been developing methods for labeling rehydrated and fresh platelets with fluorescein as well as with radioactive tags for use in hemostatic and thrombogenic studies.

In previous studies, we have used Zynaxis PKH26 to label rehydrated platelets with fluorescence. Unfortunately, this dye causes agglutination of fresh platelets. We have begun efforts to label rehydrated and fresh platelets with Zynaxis PKH2 dye, using the technique of Korash et al., who have successfully labelled fresh murine platelets with this dye (Abstract 1590, BLOOD, vol 82, No. 10, suppl. 1, Nov, 15, 1993.) Successful labeling of fresh and rehydrated platelets with this dye will allow us to monitor both fresh and rehydrated platelet infusates, providing similar control and experimental conditions.

Currently, we are working on a radiolabeling technique that will allow a 'permanent' radiolabel for infused platelets. We have investigated ^{111}Indium, ^{51}Sodium Chromate, ^{125}Iodine, and ^{131}Iodine. We have successfully labelled rehydrated platelets with ^{111}Indium. These platelets contained 90% of the label after incubation at room temperature. However, subsequent incubation of the platelets in plasma resulted in a partial release or leach of the radiolabel. We have also labelled rehydrated platelets with ^{125}I and ^{131}I with limited success. Label efficiency was approximately 50% with ^{125}I, which decreased dramatically with additional washes. ^{131}I did not label with a high efficiency. ^{51}Sodium Chromate has been used successfully for labeling fresh platelets, with little or no labeling of rehydrated platelets. We have begun investigating the possibility of labeling the platelets (with ^{51}Sodium Chromate and ^{111}Indium) prior to the fixation process, in hopes of 1)increasing the efficiency of label, and 2)stabilizing the label on the platelets. The results of these experiments will be obtained presently. The use of other radiolabelling agents such as ^{99m}Technetium and ^{51}Chromic chloride have also been considered for future use.

We have used radiolabeled autologous canine rehydrated platelets with ^{111}In for infusion studies. The labelled platelets were infused into an adult female dog. Samples were taken at 5 minute intervals for the first 45 minutes,

then once an hour until hour 6. A 2 ml whole blood sample was assayed for radioactivity at each time point. The level of radioactivity in the samples remained constant during the first 2 hours, then decreased to half the original amount after 6 hours. The possible leaching of ^{111}In label into the plasma was investigated, and might be partially responsible for the radioactivity found in the whole blood samples. At hour 4, a whole body scan of the dog was performed to discern the distribution of radioactive label. Label was found throughout the body in a diffuse distribution, with a concentration of label in the liver. Label was also detected in the major thoracic vessels. A whole body scan performed at 24 hours revealed a concentration of radiolabel in the urinary tract. Platelet counts remained within normal range throughout the experiment.

In an attempt to find other markers for fresh and rehydrated platelets, we have generated polyclonal antibodies to fresh and rehydrated human and fresh and rehydrated canine platelets. New Zealand white rabbits were immunized with platelets emulsified with Freund's complete adjuvant. Subsequent injections were performed with Freund's incomplete adjuvant every other day for 1 week. Rabbit serum was tested using ELISA and immunoblotting of platelet proteins, and by aggregation assays of fresh and rehydrated canine and human platelets. The ELISA results indicate that antibodies specific to fresh or rehydrated platelets are present in the rabbit antisera. Immunoblots confirm the presence of rehydrated human-specific antibodies. Immunoblots are currently being performed on serum from rabbits which had been injected with canine fresh or rehydrated platelets. Platelet aggregation studies indicate that serum from rabbits injected with fresh human platelets aggregate fresh human platelets with a higher efficiency and at a faster rate than rehydrated platelets. Conversely, antibodies generated to rehydrated human platelets aggregate rehydrated platelets with a higher efficiency and at a higher rate than fresh platelets. Similar results were seen in rabbits using canine fresh and rehydrated platelets. These antibodies will be used to more fully characterize surface antigens on rehydrated platelets, and as possible markers for fresh and rehydrated platelets in hemostatic and thrombogenic studies.

We are comparing the surfaces of rehydrated platelets and fresh platelets for the presence of bound protein, either plasma proteins or platelet released proteins. We have examined the platelet surface for bound plasmin, Factor XIII, and fibrinogen. Preparations of washed platelets, ACD and citrate-ACD washed platelets present all three antigens on the surface. So far, no difference has been seen between platelets stabilized with paraformaldehyde and fresh washed platelets with the bank of antibodies being used. Bound antigens had no effect on the agglutinating and clotting activity as determined in agglutinating and clotting tests in the presence of specific antibodies. We are continuing our studies on the surface antigens of the rehydrated platelets.

2. <u>The effects of rehydrated platelet transfusions on intravascular clotting (Specific aim #2)</u>

We have reported that fresh washed platelets and fixed platelets have similar plasma and platelet antigens on the surface of the both types of platelets as measured by specific antibodies. *In vitro* tests show no enhanced activation of coagulation factors with incubation of plasma and rehydrated platelets. We have also shown that lyophilized platelets generate thrombin at a faster rate than unactivated fresh platelets in the Prothrombinase Complex

reaction. Thrombin generation in that assay was dependent on the concentration of platelets. Saturation of the complex occurred with fewer rehydrated platelets than fresh platelets.

In spite of apparent increased rate of thrombin generation by rehydrated platelets, tests of plasma and serum samples collected from two dogs transfused with rehydrated platelets showed no significant change in factor VIII or FIX levels. The prothrombin times were not different from normal dogs, and there was no detectable change in fibrinogen, no significant change in thrombin clotting times, and no measurable production of fibrinogen degradation products (FDP) as measured by the Thrombo-Wellco Test. The partial thromboplastin time test (PTT), a measure of the intrinsic coagulation mechanism was slightly longer than the normal. Factor VIII assays were not conclusive and can not be repeated due to loss of sample.

In a continuation of these studies, tests of plasma and serum samples from a dog transfused seven times (see below, specific aim #3) with rehydrated canine platelets showed no significant change in factor VIII or factor IX levels. The prothrombin times for pre- and post-infusion samples were not significantly different, and no change in the production of fibrinogen degradation products was noted. The PTT was similar in pre- and post-infusion samples.

These data indicate that there is no resulting pronounced disorder of the hemostatic mechanism in dogs receiving rehydrated platelets under the conditions of our experiments. Since the Thrombo-Wellco test was negative, we assume the absence of fibrin monomer and thus no production of circulating thrombin.

3. <u>The effects of multiple infusions of rehydrated platelets in the canine animal model (Specific aim #3)</u>

In first reporting period, we reported experiments using two normal dogs which had received multiple transfusions (4 transfusions per dog for a total of 8 transfusions). Each infusate was a mixture of rehydrated platelets prepared from two unrelated dogs. Blood samples were collected from each dog pre- and post-transfusion and tested for RBC, WBC, platelets, diagnostic-multi-chem profile, electrolytes, general chemistries, and enzymes. Briefly, WBC count, respiration, and temperature varied from transfusion to transfusion. Both dogs showed evidence of allergic response after the fourth infusion that was relieved with benadryl. Neither dog received a fifth transfusion. These studies suggested that in the first dog, bovine serum albumin (BSA) was not completely removed by the washing prior to transfusion. Additional washes were done before treatment of dog 2 and no changes were noted in the dog's appearance or behavior until the fourth transfusion. Following the fourth transfusion, the dog demonstrated characteristic allergic response which was treated and relieved with benadryl injections.

The 2 animals used in this study were pups, 12-14 weeks of age. There were no remarkable differences in the chemistry profile except those seen in young dogs. Dog 1 received platelets stored in BSA and washed twice to remove albumin. 20 minutes post-infusion of platelets (10.8×10^9), the dog became lethargic and weak but recovered without treatment. We suspected contamination with BSA. The same animal was infused twice more with the same preparation of platelets but with additional washes to remove the BSA. No abnormal response was noted. However, on the fourth infusion of rehydrated platelets, the dog showed signs of discomfort and was treated and relieved by benadryl. No further infusions were given to this dog.

The second pup was treated following the same protocol as above. No reactions were noted until the fourth infusion of rehydrated platelets when a similar allergic reaction as seen with dog 1 was noted. Dog 2 was treated and relieved with benadryl and epinephrine. No further infusions were given to this dog.

Post-transfusion chemistries were not different from pre-infusion data. The most remarkable and troubling response was a drop in WBC count and a drop in platelet count immediately post infusion. This was noted in both dogs. We do not know the significance of this change. Not enough rehydrated platelets were given to significantly increase the platelet count. Since the rehydrated platelets were not labeled, we cannot know if native platelets or rehydrated platelets were sequestered.

To continue our studies on the effects of multiple transfusions of rehydrated platelets, we infused a normal adult beagle 7 times with rehydrated canine platelets which had been prepared with canine serum albumin (CSA). Each infusate was a mixture of rehydrated platelets prepared from 3 unrelated dogs. Blood samples were collected pre- and post-transfusion from the dog and tested for RBC, WBC, platelets, diagnostic multi-chem profile, electrolytes, general chemistries and enzymes. Most samples fell within normal range for the tests performed. An shallow increase in the WBC was noted after the fifth infusion, with a return to normal levels within 24 hours. This increase in WBC is thought to be attributable to contamination in the platelet preparation prior to infusion. A decrease in the neutrophil count with a concomitant increase in lymphocyte count was noted after the sixth infusion. This change in the WBC differential might be attributed to sampling error. No other significant changes in blood profiles were noted. The dog had no other visible allergic responses to the infusions, such as weakness and lethargy, which were noted in previous experiments. The respiration and temperature of the dog remained normal throughout the infusion experiment. These data suggest that rehydrated platelets prepared with CSA are tolerated in the canine animal model.